CURRENT EVIDENCE

The role of microbial exopolymers in determining the fate of oil and chemical dispersants in the ocean

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Scientific Significance Statement

Extracellular polymeric substances (EPS) are a group of chemically heterogeneous polymers released into the environment by microbes (bacteria, archaea, and phytoplankton), often in response to environmental stresses. EPS serve an important role in determining the fate and transport of oil after a spill, but relatively little is known about EPS production in relation to oil and dispersants, especially at molecular and chemical levels. Here, we summarize the scope of our current knowledge and identify major knowledge gaps.
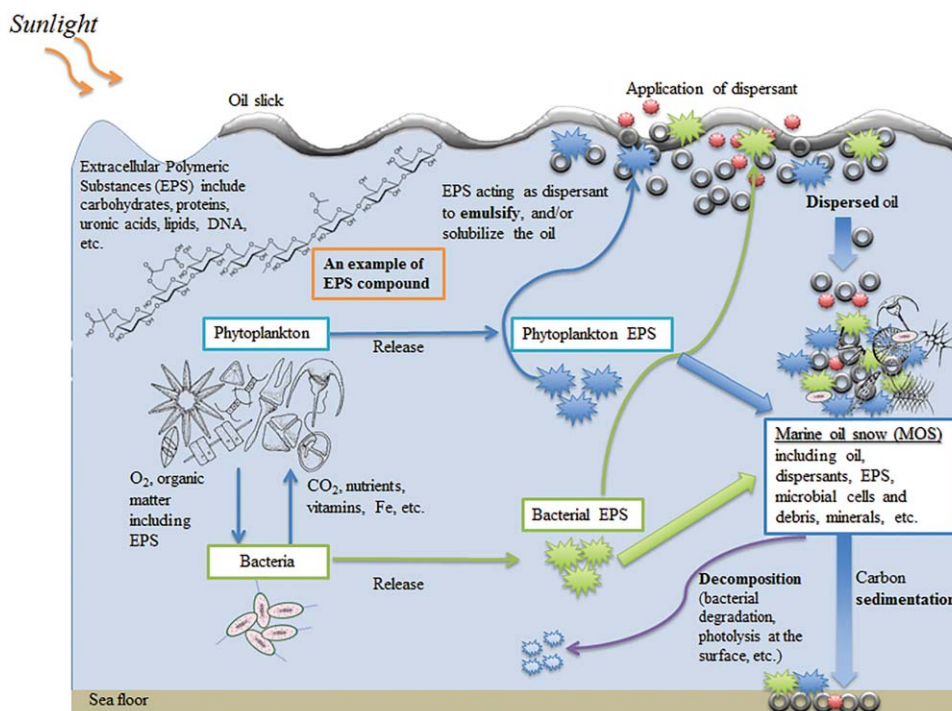
Abstract

The production of extracellular polymeric substances (EPS) by planktonic microbes can influence the fate of oil and chemical dispersants in the ocean through emulsification, degradation, dispersion, aggregation, and/or sedimentation. In turn, microbial community structure and function, including the production and character of EPS, is influenced by the concentration and chemical composition of oil and chemical dispersants. For example, the production of marine oil snow and its sedimentation and flocculent accumulation to the seafloor were observed on an expansive scale after the Deepwater Horizon oil spill in the Northern Gulf of Mexico in 2010, but little is known about the underlying control of these processes. Here, we review what we do know about microbially produced EPS, how oil and chemical dispersant can influence the production rate and chemical and physical properties of EPS, and ultimately the fate of oil in the water column. To improve our response to future oil spills, we need a better understanding of the biological and physiochemical controls of EPS production by microbes under a range of environmental conditions, and in this paper, we provide the key knowledge gaps that need to be filled to do so.

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Fig. 1. This summary figure shows the interactions between microbes, EPS, oil, and dispersant. In this figure, we give an example of an EPS molecule (typically polysaccharides, glycoprotein backbones) which is excreted by microbes—bacteria, archaea, or phytoplankton. Factors which affect the production and composition of EPS include but are not limited to nutrients, light, pH, redox, salinity as well as the presence of oil and/or dispersants. Marine oil snow (MOS) forms when compounds such as the EPS, oil, dispersant combine; but it may also contain cells or cellular debris, particulates and other materials floating in the water column. In this capacity, EPS function (1) to **emulsify** and **disperse** petroleum components, and thus enhance their solubility and bioavailability, (2) to provide physical structure allowing the microbial communities to **degrade** oil, and (3) to provide colloidal or particulate traps for petroleum components and subsequent **sedimentation** (the formation of marine oil snow) through chemical coagulation, **aggregation** and cross-linking processes.

Marine microbes are capable of producing high molecular weight exudates, called extracellular polymeric substances (EPS; also: exopolymeric substances, exopolysaccharides) (Fig. 1). The term EPS describes a heterogeneous group of materials existing in a size continuum from dissolved to colloidal phases, and includes gels, which function as particles. EPS may be attached in cell coatings, biofilms or colony matrices, or free floating. These polymeric substances have varying functional roles (e.g., protecting microbes, aiding their attachment), chemical and physical properties (Decho 1990; Hoagland et al. 1993; Decho and Herndl 1995; Leppard 1997; Verdugo 2012), and appearance (Figs. 2–5). The production of EPS by planktonic microbes can influence the fate of oil and chemical dispersants in the ocean through emulsification, degradation, dispersion, aggregation, and/or sedimentation. In turn, microbial community structure and function, including the production and character of EPS, is influenced by the concentration and chemical composition of oil and chemical dispersants. As such, EPS serve an important role in determining the fate and transport of oil after a spill, but relatively little is known about EPS production in relation to oil and dispersants, especially at molecular and

chemical levels. Oil spills remain a widespread problem, with an average 96 incidents reported per year over the last decade in the U.S.A. alone (NOAA, https://incidentnews. noaa.gov/raw/index, last accessed 09-22-2016), and marine ecosystems can take many years to show signs of recovery (Peterson et al. 2003). Characterizing the microbial processes that govern EPS production—and ultimately the fate of oil—can contribute to both explaining and predicting the response of marine systems to these catastrophic events.

Transparent exopolymer particles (TEP) are arguably the most abundant and studied form of particulate EPS in the oceans (Passow 2002), in part because they are extremely surface active (Mopper et al. 1995; Zhou et al. 1998) and are the underlying matrix of marine snow (e.g., Alldredge et al. 1993; Passow et al. 1994; Kiørboe 2001; Simon et al. 2002), which drives particle sedimentation (e.g., see Fig. 2). Transport of particles to the bottom of the ocean via marine snow is a key pathway of the carbon cycle (Alldredge and Gotschalk 1988, 1989; Asper et al. 1992; Burd and Jackson 2009; Fu et al. 2014; Daly et al. 2016). The microbial release of EPS in aquatic environments may be thought of as part of an autopoietic system, that is, a self-sustaining community response (Varela et al. 1974;
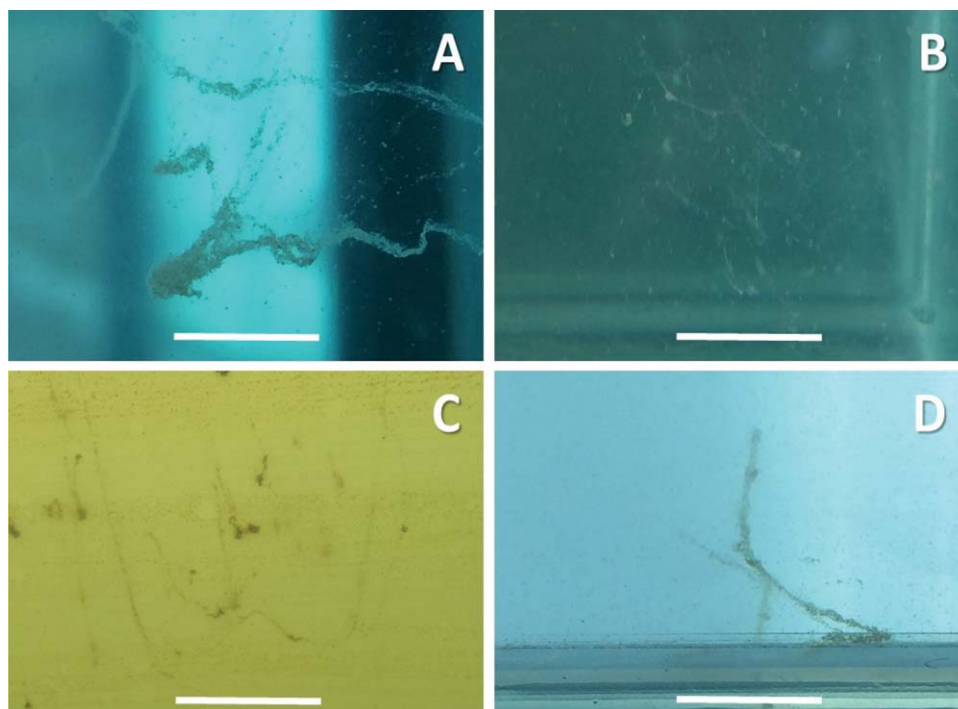
Fig. 2. Mesocosm experiments were performed as part of the ADDOMEx GOMRI funded program with seawater collected from the NGOM and "seeded" with microbial populations collected from Galveston Bay (Texas). Four treatments were set up in triplicate (**A**) Control, (**B**) water accommodated oil fraction, (**C**) water accommodated oil fraction with Corexit 9500, and (**D**) a 10-fold diluted equivalent of (**C**). Bars represent 2 cm.

Maturana and Varela 1980; Santschi et al. 2003). If this is indeed the case, then EPS production is of great ecological importance. The processes, mechanisms, and participants however are poorly known and require further study.

The physical-chemical mechanisms by which EPS enhance aggregation and/or dispersion of oil need to be better understood. Additionally, it is important to investigate how the presence of dispersants affect mechanisms leading to the sedimentation of oil in a ternary system (oil-dispersant-EPS), compared to that in a binary system (oil-EPS) (Fig. 6). EPS have an important role in determining the fate and transport of oil after a spill, but relatively little is known about EPS production in relation to oil and dispersants, especially how the amphiphilicity and surface activity of EPS are controlled at molecular and chemical levels. In this article, we review what we do know about microbially produced EPS, how oil and chemical dispersant can influence the production rate and chemical and physical properties of EPS, and ultimately the fate of oil in the water column. In addition, we discuss this work in the context of the Deep Water Horizon (DwH) oil spill because it is the first such event in which the importance of marine oil snow (MOS), a biological process, as a transport pathway for oil to the seafloor, was observable. The scale of this production of MOS clearly has an important impact of the ecosystem, its alterations and/or recovery, and will do for some time to come.

Background on the study of marine snow and oil spills

Interest in the significance of marine snow can be traced back to observations from the 1800s when EPS-rich marine snow was considered a potentially important food source for deep-sea animals (see review by Silver 2015). Pioneering studies of "suspended, mucous-rich particles, containing abundant microbial populations" however did not begin until the 1940s and 1950s (Silver 2015). Silver et al. (1978) was the first to quantify, in situ, both the abundance and microbial community composition of marine snow particles, revealing them to be a "hot spot" of microbial activity (see also Fig. 3). Later work found that a majority of carbon cycling in the sub-euphotic zone occurs within marine snow, which have elevated bacterial production and exoenzyme activity compared to free-living microbes in the water column (Cho and Azam 1988; Smith et al. 1992; Ziervogel et al. 2012; Arnosti et al. 2016). More recently, EPS have been shown to be a major reservoir of organic carbon (ca. 10–25%) in the ocean, with an estimated global pool of ~ 70 Gigatons, or more than two times the total amount of living biomass (Hansell and Carlson 2001; Verdugo et al. 2004; Verdugo and Santschi 2010). In addition, there has been a renewed interest in better understanding marine snow because of its perceived role in influencing the fate of both oil and dispersants in marine environments.

For example, during the Deepwater Horizon (DwH) oil spill in the Northern Gulf of Mexico (NGOM), different types
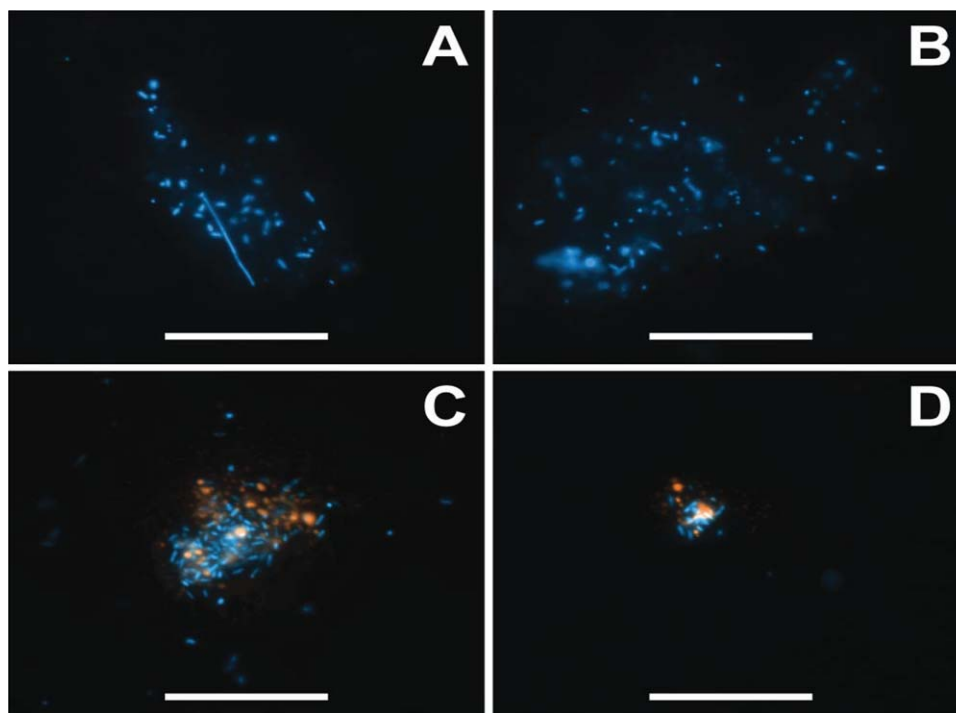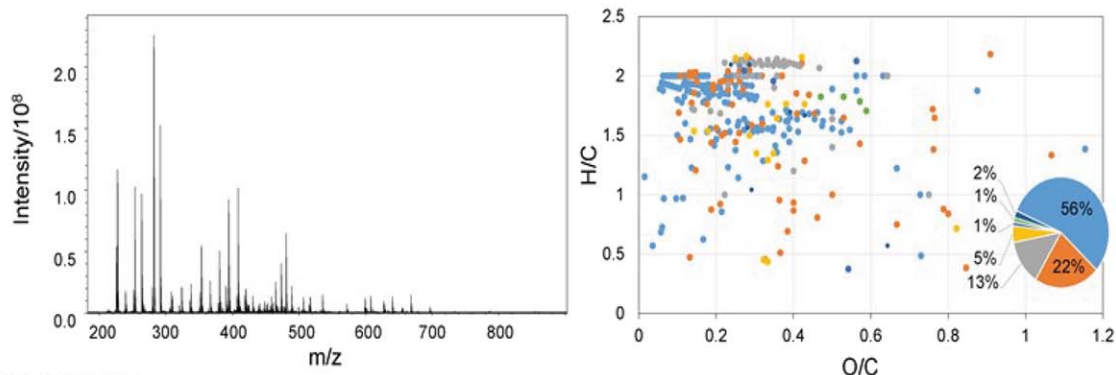
Fig. 3. Mesocosm experiments were performed (as part of the ADDOMEx GOMRI funded program) with seawater collected from the NGOM and "seeded" with microbial populations collected from Galveston Bay (Texas). False-colored photographs of micro-scale microbial aggregates collected from four different mesocosm treatments after 48 h. (**A**) Control, (**B**) water accommodated oil fraction, (**C**) water accommodated oil fraction with Corexit 9500, and (**D**) a 10-fold diluted equivalent of (**C**). Water samples were fixed with formalin (2% v/v final concentration), stained with DAPI (45 μM final concentration), and visualized on a Zeiss Axio Imager 2 microscope. Bars represent 20 μm. The DAPI-stained cells appear blue, while oil /Corexit appears orange. In (**B**), no oil droplets or globules were observed.

of marine snow formed that incorporated oil (Passow et al. 2012; Ziervogel et al. 2012; Daly et al. 2016). It has been suggested that the formation of this MOS (Passow et al. 2012; Daly et al. 2016; Passow and Hetland 2016) ultimately resulted in 4–31% of the DwH oil being returned back to the seafloor or deposited on nearby corals as a loose flocculent (White et al. 2012; Valentine et al. 2014; Chanton et al. 2015, Passow and Ziervogel in press). Mucus-rich MOS differed from aggregates containing oil, in that it formed in the absence of particles other than bacteria. Like a biofilm, MOS consists of a matrix of bacterial colonized EPS, whereas aggregates include diverse particles (e.g., algae) embedded in an EPS matrix. In this way, EPS arguably served not only as a vehicle for the transport of oil to depth via EPS-rich marine snow, but also acted as a natural dispersant. Its amphiphilic properties—possessing both hydrophilic (*water-loving, polar*) and lipophilic (*fat-loving, non-polar*) characteristics—increased the interfacial area between oil and microbes and thus enhanced its biodegradation (e.g., Kappell et al. 2014; Ron and Rosenberg 2002; Head et al. 2006; Quiroz et al. 2006; Ding et al. 2008, 2009; McGenity et al. 2012; Gutierrez et al. 2013; Daly et al. 2016; Joye et al. 2016; Passow and Ziervogel in press).

Microbes (bacteria, archaea, and phytoplankton) that are capable of conducting oil degradation are ubiquitously found in marine waters, but typically represent only a small fraction of the pre-spill communities (Head et al. 2006; Valentine et al. 2014; Baelum et al. 2012). The release of petroleum hydrocarbons, which includes polycyclic aromatic hydrocarbons (PAHs) and other petrocarbons, triggers a complex cascade of microbial responses. No single species dominates, but instead microbial consortia develop (MacNaughton et al. 1999; Kappell et al. 2014; Head et al. 2006; Baelum et al. 2012; Joye et al. 2016), with EPS forming the matrix of microbial aggregates and are functionally comparable to biofilms. Further, tiny oil droplets trapped in the EPS matrix are available to the microbial communities in the aggregates (Gutierrez et al. 2013; Fig. 3B–D). During the DwH oil spill which lasted 3 months, the bacterial community response was dominated by a large bloom of hydrocarbon-degrading Gammaproteobacteria. This bloom included abundant populations of alkane-degrading bacteria from the order *Oceanospirillales* and obligate PAH degraders of the genus *Cycloclasticus* (Kappell et al. 2014; Hazen et al. 2010; Baelum et al. 2012; Mason et al. 2012; Dubinsky et al. 2013; Rivers et al. 2013; Joye et al. 2016). Other microbial groups found in the post-spill community included various members of the metabolically versatile *Roseobacter* clade as well as moderately psychrophilic members of *Colwellia* with

A) Control EPS

B) WAF EPS
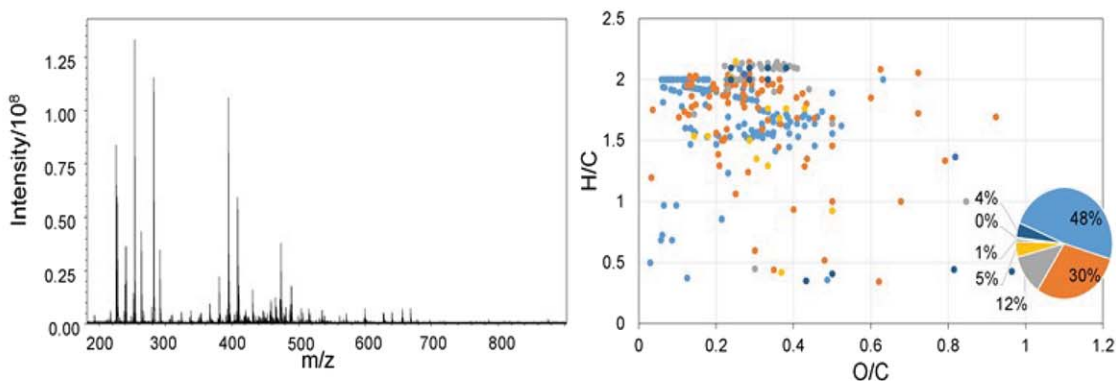
C) DOM
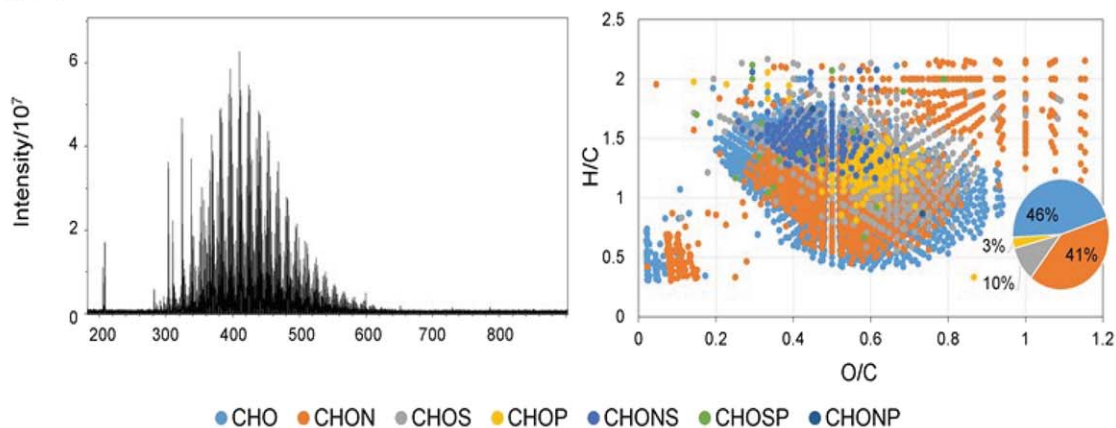
● CHO ● CHON ● CHOS ● CHOP ● CHONS ● CHOSP ● CHONP

Fig. 4. Mesocosm experiments were performed as part of the ADDOMEx GOMRI funded program with seawater collected from the NGOM and "seeded" with microbial populations collected from Galveston Bay (Texas). Of the treatments, negative mode Electrospray Ionization Mass Spectrometry Coupled Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FTICR-MS) spectra (left) and their associated Van Krevelen diagrams (right) of (**A**) pyridine extract of marine snow collected from a control mesocosm tank, (**B**) pyridine extract of marine snow collected from a WAF mesocosm tank, and (**C**) bulk DOM from a control mesocosm tank. Pie chart inserts denote percentages of molecular formulas containing CHO, CHON, CHOS, CHONS, CHOSP, and CHONP.

the ability to degrade a wide range of hydrocarbon compounds (Prince et al. 2010; Redmond and Valentine 2012; Arnosti et al. 2016; Yang et al 2016). Archaeal communities were dominated by *Euryarchaeota* or *Thaumarchaeota* consistent with non-spill conditions, but the relationships

between archaea and oil remain difficult to resolve and require further study (Redmond and Valentine 2012). Less is known about the role of eukaryotic autotrophs in the degradation of oil, but some species, like the diatom *Skeletonema* sp. were abundant in sediment traps after the spill
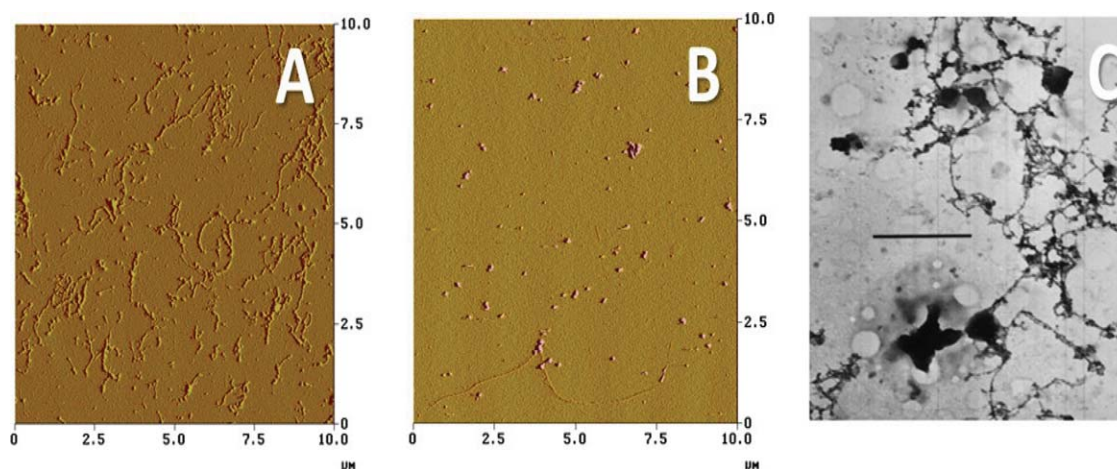
Fig. 5. Fibrils in colloidal organic matter (1–3 nm thickness and 100s to 1000s of nm in length) from the Middle Atlantic Bight, taken at 2 m and 2600 m water depth, which had modern radiocarbon ages when composed of 100% carbohydrate (Santschi et al. 1998; with permission from Wiley Rightslink® by Copyright Clearance Center); (**A**) and (**B**) imaged by Atomic Force Microscopy, after mounting on mica, with image size of 10 × 10 μm; (**C**) imaged by Transmission Electron Microscopy, after staining with Ru oxychloride, and mounted on hydrophilic nanoplast resin. Image bar is 500 μm. All images show the "spiderweb" fibrillar structures appearing as pearls on necklaces; fibrillar forms of colloids were only found in surface and bottom waters, but not in mid-depth waters.

(Yan et al. 2016), and are commonly associated with oil residues in sediments (Parsons et al. 2014).
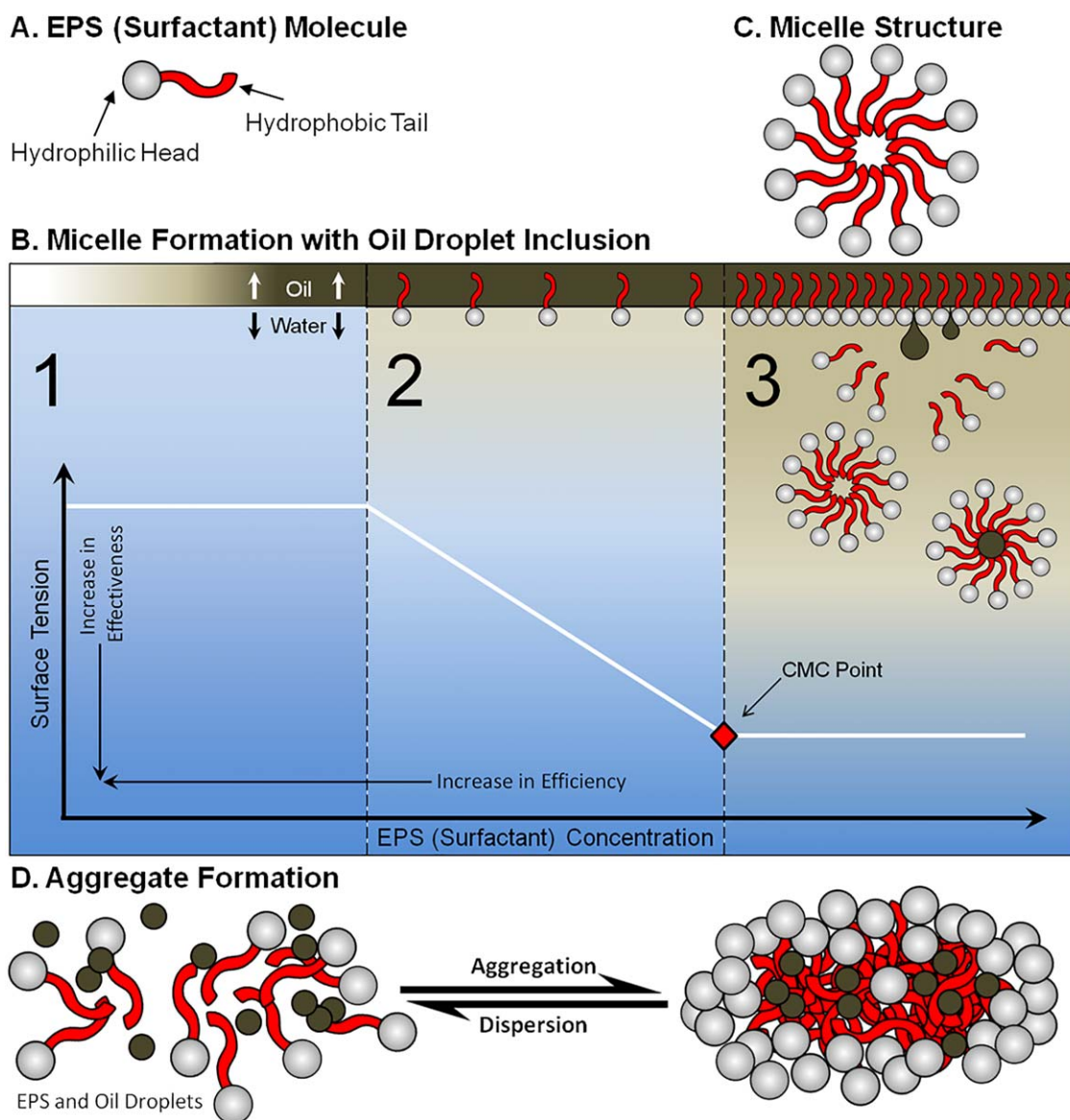
Extracellular polymeric substances
Operational definitions of EPS

EPS, extracellular polymeric substances, are "operationally" defined based upon their characteristics, size(s), and methods of quantification (Table 1). Exopolymeric substances released by cells (e.g., polysaccharides, proteins) are typically nanofibers (Leppard 1997; Santschi et al. 1998) which self-assemble into colloidal sized nanogels (Chin et al. 1998; Verdugo et al. 2008; Orellana and Leck 2015) that are highly organized crystalline-ridged threads, with widths of 1–2 nm and lengths of several μm (Fig. 5). Nanogels form larger gels (micro-gels) and porous networks (Passow 2000; Verdugo 2007; Verdugo and Santschi 2010). Transparent exopolymer particles, TEP, are an operationally defined group of particles that form from dissolved precursors (see text box) and have the properties of gels (Bar-Zeev et al. 2015). In the ocean, self-assembly of gel particles (nano-or microgels) from colloidal EPS (e.g., Verdugo et al. 2004; Verdugo and Santschi 2010) is rapid, i.e., it only takes hours to days (Chin et al. 1998; Ding et al. 2008, 2009), and is dependent on temperature, salinity, and pH (Chen et al. 2015) as well as ultraviolet (UV) radiation (Orellana and Verdugo 2003) (see also Fig. 6). TEP, which are retained on a polycarbonate filter (usually > 0.4 − 100's μm's; but per definition exclude coatings, or colony matrixes) are stained with Alcian blue, a dye specific for acidic polysaccharides. The amount of dye bound to TEP may be determined colorimetrically and TEP concentrations expressed as gum xanthan equivalents (Alldredge et al. 1993; Passow and Alldredge 1995). When calibrated with alginic acid, the stained particles

have also been called acid polysaccharide particles (APS), with results dependent on reference compound (Hung et al. 2003; Santschi et al. 2003). TEP precursors (Thornton 2002) or specifically colloidal TEP (cTEP, 0.05 μm < particle size < 0.4 μm; Villacorte et al. 2009), may be distinguished separately (see text box). Coomassie stained particles (CSP) are proteinaceous particles made visible with the dye Coomassie blue, and exhibit a different dynamic than TEP (Long and Azam 1996; Nagasaki et al. 2004; Verdugo et al. 2008; Cisternas-Novoa et al. 2015). Marine snow (Figs. 2, 5), defined as composite particles > 0.5 mm, consists of a gel matrix and solid particles like cells (bacteria, phytoplankton), feces (zooplankton), detritus and/or minerals (Alldredge and Silver 1988, 1989; Asper et al. 1992; Diercks and Asper 1997; Pilskaln et al. 1998; Armstrong et al. 2009). When EPS concentrations are high, marine snow may be distinctly different in appearance from diatom aggregates or fecal matter. Figure 2 provides examples of some of the differences possible. It is not well understood how the differently defined forms and types of EPS are related to each other (see also Bar-Zeev et al. 2015). This makes the task of identifying their relationships and interactions extremely complex and challenging. Below we make an effort to do so.

Chemical composition of EPS

Chemically, EPS consist largely of acidic polysaccharides and proteins that occur in the form of glycoproteins, proteoglycans, glycolipids, uronic acids and other macromolecules including DNA and enzymes (Azam 1998; Verdugo et al. 2004; Verdugo 2007; Zhang et al. 2008; Xu et al. 2011a,b; Zhang and Santschi 2009; Verdugo and Santschi 2010). In the colloidal (but not marine snow sized) fraction of EPS, only 6–18% of carbohydrates are acidic polysaccharides

A. EPS (Surfactant) Molecule

C. Micelle Structure

B. Micelle Formation with Oil Droplet Inclusion

D. Aggregate Formation

Fig. 6. (**A**) shows the amphiphilic nature of an EPS molecule as a surfactant with a hydrophilic head group and a hydrophobic tail. (**B**) Demonstrates three stages of interfacial tension (IFT) as a function of EPS surfactant concentration, where (**Phase 1**) is the highest IFT at the interface between oil/water and little or no EPS; (**Phase 2**) an increasing concentration of EPS molecules are diffusing to the interface and are thus decreasing the IFT between the oil and water interface; (**Phase 3**) At the onset of this stage, the interfacial surface has become saturated with EPS molecules and the critical micellar concentration (CMC point) is reached, where micelles, (**C**), form. (**C**) is a representation of a micelle with the EPS biosurfactants oriented in a fashion that solubilizes and entrains oil, organic matter, and hydrophobic portions of EPS molecules while still trapping water within, emulsifying and dispersing the trapped material or, (**D**), aggregating into networks of gels.

(including uronic acids; Guo et al. 2002), whereas acidic polysaccharides dominate the $< 0.45 \ \mu m$ fraction, and specifically the colloidal fraction which can be captured by a 1 kDa ultra filtering membrane (Zhang et al. 2008; Xu et al. 2009). Total carbohydrates (neutral plus charged) can make up 40% (or less) of the total particulate organic carbon content of EPS (Guo et al. 2002; Hung et al. 2003; Santschi et al. 2003; Xu et al. 2011*a,b*). Of the polysaccharides associated with marine algae and bacteria in the surface waters in

the NGOM after the DwH spill, pullulan [α(1,6)-linked maltotriose(glucose)], laminarin [β(1,3-glucose)], xylan (xylose), fucoidan (sulfated fucose), arabinogalactan (arabinose and galactose), and chondroitin sulfate (sulfated N-acetylgalactoseamine and glucuronicacid) were measured in aggregate-oil water-ambient water combinations by Arnosti et al. (2016). In culture, phytoplankton have been found to produce highly diverse and complex sugars, primarily pentoses, hexoses, 6-deoxyhexoses, *O*-methylated sugars, and

Table 1. Operationally defined types of exopolymeric substances with corresponding size, characteristics, and methods using to identify them (primary, but not only methods), and their known abundances in the ocean.

Type (EPS)	Size	Characteristics /composition	Identification method	Abundance*	References
Nanofibers	< 1 μm		AFM		Leppard (1997), Santschi et al. (1998)
Nanogels	< 1 μm		DLS; AFM		Chin et al. (1998), Verdugo (2012)
Microgels	1–10 μm	Proteins, polysaccharides, lipids, DNA	DLS; flow cytometer	$\sim 3 \times 10^6 - 10^{12}$/L	Chin et al. (1998), Verdugo et al. (2008), Verdugo (2012)
Colloidal TEP	> 0.05 μm and < 0.4 μm	Acidic polysaccharide, associated with diverse other substance classes and trace elements	Filtration and Alcian blue stain		Villacorte et al. (2009)
TEP	Operationally defined > 0.2 or 0.4 μm	Acidic polysaccharide, associated with diverse other substance classes and trace elements	Filtration and Alcian blue stain	$\sim 10^3 - 10^9$/L	Alldredge et al. (1993), Passow and Alldredge (1995), Passow (2000,2002), Verdugo (2007), Verdugo and Santschi (2010)
CSP	Operationally defined	Proteins, presumably associated with diverse other substance classes and trace elements	Coomassie blue stain	~ 2–3 $\times 10^7$/L	Long and Azam (1996), Nagasaki et al. (2004), Verdugo et al. (2008), Cisternas-Novoa et al. (2015)
Marine snow	> 0.5 mm	Composite particles (algae, minerals, feces, detritus…) encased in an EPS matrix:	Macroscopically visible, cameras, optical microscopy	$\sim 5 \times 10^{-4} - 5 \times 10^2$/L	Alldredge and Silver (1988), Santschi et al. (1999)
MOS	> 0.5 mm	Oil associated marine snow	Macroscopically visible, cameras, optical microscopy		Ziervogel et al. (2012), Passow et al. (2012), Passow (2016), Daly et al. (2016), Passow and Ziervogel (2016)

*Abundance numbers are intended to be "representative" as they are known to be highly variable.

aminohexoses (e.g., Hoagland et al. 1993; Chiovitti et al. 2003). It is because of their high polysaccharide content, mainly cross-linked with divalent Ca^{2+} cations, that these substances have polymeric characteristics, with their extracellular occurrence leading to their designation as exopolymeric substances.

There is some preliminary evidence that the protein/carbohydrate ratio of EPS exerts control on their hydrophobicity, surface activity and aggregate formation (Stenstörm 1989; Liao et al. 2001, 2002; Dickinson 2003), but the wider applicability of this concept remains to be shown. For example, EPS, which contain a substantial amount of more hydrophobic protein (Xu et al. 2011a,b; Zhang et al. 2012), has been shown to be responsible for the faster and nonionic aggregation behavior of some marine gels (Ding et al. 2008, 2009; Chen et al. 2011). In Table 2, measured bacteria and phytoplankton EPS protein/carbohydrate ratios, and their corresponding hydrophobic contact area (relative hydrophobicity and amphiphilicity) are summarized. We found a significantly positive correlation between the protein/carbohydrate ratio and hydrophobic contact area ($p < 0.01$). This suggests that the relative hydrophobicity of EPS, which can be responsible for gel formation, particle aggregation and bioflocculation, are regulated, at least in

Table 2. Examples of the composition of characterized bacterial and phytoplankton EPS. "Attached" EPS are that which adheres to cells and are more hydrophobic than "non-attached" EPS, which is free-floating in seawater.

	Species	EPS	Protein/CHO	Hydrophobic contact area (Å)	Ref.
Bacteria	*Pseudomonas fluorescens* Biovar II	Attached	0.45	58.5	Xu et al. (2011*b*)
	Pseudomonas fluorescens Biovar II	Non-attached	0.05	18.3	Xu et al. (2011*b*)
	Sagittula stellata	Attached	0.18	13.9	Xu et al. (2011*b*)
	Sagittula stellata	Non-attached	0.12	11.2	Xu et al. (2011*b*)
Phytoplankton	*Amphora* sp.	Non-attached	Below detection limit	75.2	Zhang et al. (2012)
	Thalassiosira pseudonana	Attached	2.22	86.4	Zhang et al. (2012)
		Non-attached	0.67	79.4	Zhang et al. (2012)
	Dunaliella tertiolecta	Attached	1.02	85.6	Zhang et al. (2012)
		Non-attached	0.75	72.0	Zhang et al. (2012)
	Phaeocystis globasa	Attached	1.55	n.d.	Zhang et al. (2012)
		Non-attached	0.22	n.d.	Zhang et al. (2012)
	Phaeodactylum tricornutum – with Si in media	Attached	0.93	n.d.	Chuang et al. (2014)
	Phaeodactylum tricornutum – with NO Si in media	Attached	0.50	n.d.	Chuang et al. (2014)

part, by relative protein/carbohydrate ratios. Further, these studies found that "attached" or "capsular" EPS (i.e., that which adheres to cells) are usually more hydrophobic than "non-attached" EPS (i.e., dissolved EPS that are free-floating in the water) (Xu et al. 2011*a,b*; Zhang et al. 2012), with the former usually having a higher protein/carbohydrate ratio than the latter. The increase in this ratio has been correlated with a decrease in the negative surface charge which in turn favors aggregate formation (Wang et al. 2006).

EPS production

Both prokaryotes and eukaryotes generate EPS abundantly. Marine bacteria generally produce EPS with higher levels of uronic acids (Table 2) compared to those found in EPS produced by marine eukaryotic phytoplankton and non-marine bacteria (Kennedy and Sutherland 1987; Ford et al. 1991; Bhaskar and Bhosle 2005). The production rate and composition of EPS is also dependent on environmental conditions. Nitrogen and phosphorus limitation, light regime and temperature have all been shown to be important (Mague et al. 1980; Myklestad et al. 1989; Myklestad 1995; Staats et al. 2000; Urbani et al. 2005). Additionally, interactions between eukaryotic phytoplankton and associated bacteria alter production of EPS, especially TEP (Grossart 1999; Grossart et al. 2006; Gärdes et al. 2011; Seebah et al. 2014). Gaerdes and co-authors (2011), for example, found that TEP production was reduced in the absence of either live *Marinobacter adhaerens* HP15 (bacteria) or live *Thalassiosira weissflogii* (diatom) compared to treatments where both were present and alive. Some forms of EPS act as high nutrient substrates for microbes (Azam 1998; Verdugo et al. 2004; Azam and Malfatti 2007; Verdugo and Santschi 2010) and differences in its composition and characteristics will lead to

variations in its nutritional value. EPS have also been shown to act as a non-specific ligand for iron and other trace elements, thereby potentially enhancing phytoplankton growth (Steigenberger et al. 2010; Strmečki et al. 2010; Hassler et al. 2015; Quigg 2016). The effectiveness of EPS in scavenging trace elements and nanoparticles, may also benefit microbes by protecting them from toxic trace metal ions (e.g., Cu) and/or nanoparticles (Chen et al. 2011; Zhang et al. 2012, 2013; Quigg et al. 2013).

Relatively little is known about how oil and dispersants influence EPS production in phytoplankton. Lab studies indicate the controls and types of EPS may be group and species-specific. For example, diatoms (planktonic and benthic) produce a number of structures using EPS including stalks (uni-directionally deposited, multilayered structures), tubes (structured pseudofilaments), apical pads (small globular structures attaching cells to each other), adhering films, fibrils (Fig. 5), and cell coatings or colony matrices, e.g., in *Chaetoceros socialis* (Drum 1969; Hoagland et al. 1993). While stalks and apical pads help diatoms attach to substrata, tubes and capsules enclose cells. Other roles for EPS in diatoms include motility, habitat stabilization and colony formation (e.g., microphytobenthic communities found along sand flats), and/or anti-desiccation (find more detail in Hoagland et al. 1993; Thornton 2002). Pelagic diatoms release colloidal EPS (Myklestad et al. 1989; Decho 1990; Leppard 1997), some of which form TEP, possibly to physiologically balance energy with carbon and nutrient acquisition (Passow and Laws 2015) and possibly as a lifecycle strategy to enhance sedimentation and survival (Smetacek 1985).

Coccolithophores, a globally distributed calcifying group of phytoplankton, produce EPS and TEP (Engel et al. 2009;

Biermann and Engel 2010). Calcification in coccolithophores requires a layer of acidic polysaccharides encapsulating the cell for production and attachment of coccoliths (de Jong et al. 1979). Coccoliths are embedded in this acidic polysaccharide layer, which is rich in uronic acid that facilitate Ca^{2+} binding (de Jong et al. 1976; van Emburg et al. 1986). The capsule of acidic polysaccharides will stain with Alcian blue in calcifying strains of the coccolithophore *Emiliania huxleyi*. Polysaccharides from this capsule slough off forming free TEP (Engel et al. 2004). Coccolith shedding by *E. huxleyi* typically occurs at the end of bloom events, in particular following nutrient limitation, viral infection, or changes in light availability (Engel et al. 2009). Given how variable EPS production is between taxa, the influence of oil and dispersants is likely dependent upon the composition of the microbial community. While some laboratory-based studies have investigated these responses, much more work is needed to characterize species-specific interactions with these chemicals.

EPS-marine gels

Marine gels consist of three dimensional cross-linked polymers with seawater as the solvent. Marine gel networks are stabilized by physical entanglement of polymers, such as the formation of Ca^{2+} bridges and are sometimes enhanced by hydrophobic interactions (Chin et al. 1998; Orellana et al. 2003; Ding et al. 2008, 2009). The behavior of nanogels, microgels, and TEP (Table 1) may be explained using gel theory (TEP specifically will be discussed in more detail below) (Chin et al. 1998; Verdugo et al 2004; Orellana and Leck 2015). Due to their unique network structure, the gel mass is mostly liquid, but can behave as sticky solids (Orellana and Leck 2015). Characteristics of marine gels include spontaneous assembly within hours to days, volume phase transition at specific pH or temperature points and dispersion following Ca^{2+} chelation (Chin et al. 1998; Orellana et al. 2007; Verdugo 2012; Chen et al. 2015). TEP are composed of acidic polysaccharide-rich macro-gels, that form from dissolved precursors within hours (Passow 2000), change size due to changes in pH (Mari and Robert 2008) or temperature (Piontek et al. 2009; Seebah et al. 2014), and disperse when Ca^{2+} are removed using EDTA (Alldredge et al. 1993). UV radiation cleaves polymers and inhibits marine gel assembly, or even disperse assembled gel networks (Orellana and Verdugo 2003), including TEP (Ortega-Retuerta et al. 2009). Temperature and pH changes also can reduce marine gel formation and induce dispersion (Chen et al. 2015).

About 10–30% of marine dissolved organic carbon self-assembles spontaneously and reversibly in a two-step process (Verdugo and Santschi 2010; Verdugo 2012; Orellana and Leck 2015). Microbial degradation and UV dispersion can result in shorter polymer chains that lead to gel structure instability and low bioavailability. The bioavailability of small molecules (< 600 Da) is high as bacteria may utilize them directly. Dissolved organic matter polymers that cannot be directly incorporated into the cell, can become bioavailable in gel form that act as hotspots for microbial activity. However, polymers that are too large for direct uptake, but fall short to form stable gels, will remain less bioavailable in the DOM pool (Verdugo 2012; Orellana and Leck 2015). Hydrophobic interactions have been shown to significantly impact the gel assembly process (Ding et al. 2008, 2009; Chen et al. 2011, 2015; Orellana et al. 2011). The addition of oil dispersants, which are rich in hydrophobic and hydrophilic moieties (detergents), is expected to disperse gels, especially those where hydrophobic binding plays a major role in driving gel formation (see Fig. 6). Further, the role of oil in stimulating or impeding this process is under investigation.

EPS-transparent exopolymer particles

TEP as "physical gels" are stabilized by physical entanglements and weak hydrophobic interactions and can easily assemble and disperse (Verdugo and Santschi 2010; Verdugo 2012). The interactions need little activation energy (< 50 kJ mol^{-1}) and are reinforced by Ca^{2+} bridges, which cross-link ionized carboxyl groups (negatively charged) in neighboring TEP chains (Chin et al. 1998), and follow along a thermodynamically favorable assembly process (Verdugo 2012). "Chemical gels" formed along the biotic pathway are less common and characterized by covalently cross-linked, irreversibly attached biopolymers (Verdugo 2012; Bar-Zeev et al. 2015). This pathway involves direct sloughing of mucus from cell capsules or colony matrices of phytoplankton or bacteria. This TEP is frequently interlinked by strong covalent bonds (400 kJ mol^{-1}) and assembly is ordinarily irreversible (Verdugo 2012).

TEP occurs as free-floating gel particles that may form abiotically and spontaneously from dissolved precursors, or biologically when colony matrices disintegrate (e.g., from *Chaetoceros socialis* or *Phaeocystis* spp.; Passow and Wassmann 1994). Abiotic formation constitutes the predominant "physical pathway" where TEP forms from dissolved fibrillar polysaccharides released by planktonic organisms (Passow 2000, 2002, 2012; Verdugo and Santschi 2010; Verdugo 2012; Villacorte et al. 2013). TEP and their "non-particulate" precursors exist in a dynamic equilibrium with TEP (Verdugo et al. 2004). Increased release of TEP may occur during or after algae blooms and localized stress conditions, wherein the decoupling of nutrient availability from energy and carbon supplies may lead to an escalation in TEP release (Passow and Laws 2015). *Thalassiosira pseudonana*, for example, releases large quantities of TEP during the end stage of a bloom when nutrients are becoming limited, corresponding to physiological signs of stress such as decreased photosynthetic efficiency and growth rates (Kahl et al. 2008). Similarly, an increase in the total TEP pool was observed in

cultures of the diazotrophic cyanobacteria *Trichodesmium* in response to Fe limitation (Berman-Frank et al. 2007). In coccolithophores, coccolith shedding, associated with stress, increases TEP concentrations and coagulation (Engel et al. 2004; Chow et al. 2015). Large accumulations of TEP may also lead to fish kills as it can smoother the gills and suffocate animals (as observed in McInnes and Quigg 2010), or may lead to large foam events after *Phaeocystis* blooms (Riegman et al. 1992), or to huge mucus events in the Adriatic (Stachowitsch et al. 1990).

During aggregation, TEP provides the glue and matrix to hold particles together (Alldredge et al. 1993). Aggregation rates primarily depend on the size, concentration and stickiness of particles (Jackson 2005; Burd and Jackson 2009). The stickiness is defined as the probability that two particles adhere after they have collided (Jackson 1990). TEP stickiness (typically between 0.05 and 0.8) is orders of magnitude higher than the stickiness of other marine organic particles (typically ≤ 0.01) and thus usually dominates aggregation rate (Mari et al. 2014). However, TEP stickiness varies substantially and the relationship between stickiness and chemical properties of TEP are largely unidentified. Older TEP, which is thought to be modified by bacteria, is stickier than fresh TEP produced by autotrophs (Rochelle-Newall et al. 2010). High concentrations of trace metals reduce TEP stickiness (Mari and Robert 2008). In order to estimate the carbon content in TEP, various conversion factors have been empirically determined (Mari 1999; Engel and Passow 2001); conversion factors for natural samples are currently being determined. Given how much the properties of TEP can vary with environmental conditions, more work is needed to understand the modifications imposed by oil and dispersant exposure. This is key in developing a better understanding of the role of TEP in carbon cycling.

EPS-marine snow

In the presence of solid particles, TEP promote the formation of sinking aggregates because of its high stickiness (Logan et al. 1995; Mari et al. 2014), but in the absence of ballasting particles, TEP accumulates in the sea surface microlayer (Wurl et al. 2009). Marine snow (Figs. 1–4) is central for the transport of organic matter to the deep sea. Only particles large enough to sink rapidly reach the deep ocean where they provide food for heterotrophs and lead to the sequestration of carbon, i.e., its removal from the atmosphere for > 100 yr. Marine snow frequently accumulates at fronts or in thin layers, especially at sharp density gradients formed by differences in salinity and other hydrographic parameters (references in Turner 2015) and is ubiquitous after the peak phase of many diatoms blooms (Kiørboe et al. 1994; Thornton 2002; Burd and Jackson 2009). Marine snow is formed either by physical coagulation (collision and attachment) of particles into aggregates or by zooplankton activity (Alldredge and Silver 1988; Jackson 1990; Kiørboe

2001; Simons et al. 2002). The formation of MOS via disproportionate mucus production by bacteria in response to oil, has recently been described (Passow 2016).

Towards identifying factors that determine the chemical properties of marine snow, especially of the TEP mucus matrix, we recently examined marine snow chemically (Chen Xu, unpubl.) and spectrally using electrospray ionization mass spectrometry coupled to Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). This process identified between 300 and 400 individual molecules in the pyridine extracted marine snow fraction (which includes microbial cells and extracellular material) collected from the NGOM (Fig. 4A) and from a water accommodated oil fraction (WAF)-amended NGOM (Fig. 4B). While this number is relatively high; it is far less than that observed in the dissolved organic matter extracted from the same NGOM sample (Fig. 4C). The number of peaks assigned to the various types of elemental formulas (CHO, CHON, CHOS, etc.) are represented as pie chart inserts to each plot. These help us to understand the variety of negatively charged (e.g., carboxyl, phosphoric, sulfate, and hydroxyl) and positively charged (e.g., amino) functional groups biopolymers carry. The dominant formulas are those which contain only CHO elements attributable to lipids (high H/C and low O/C) in the region of the van Krevelen diagram (Fig. 4). The next most abundant formulas are associated with proteins: CHON formulas that appear to be mostly concentrated in the protein-like region of the van Krevelen diagram and those containing S atoms probably associated with S-containing proteinaceous substances. It is worth pointing out that the various extraction methods (Xu et al. 2009; Zhang and Santschi 2009) tested for their ability to maintain cell integrity while releasing most attached EPS (Chen Xu, unpubl.) suggest that 1% EDTA is sufficient to remove this material from algae and bacterial cells without cell damage. Nevertheless, these preliminary results indicate that pyridine extracts of marine snow are dominated by molecules mostly associated with biological organisms.

EPS interactions with petrocarbons and hydrocarbons

The DwH spill released "live oil" into the environment, that is, a mixture of oil and natural gas with very high vapor pressures. Oil exposed to normal temperature and pressures loses its gaseous hydrocarbons (natural gas) and is called "dead oil" (Reddy et al. 2012). In situ burning of oil releases pyrogenic hydrocarbons, which differ appreciably from crude oil (Overton et al. 2016). Moreover, the presence of oil may range from thick slicks to thin sheens at the surface to the WAF dispersed within the water body. The water soluble fraction of oil ($< 1\%$), and its derivative products are complex mixtures of alkanes, cyclohexanes, monoaromatic hydrocarbons (e.g., benzene, toluene, xylenes) and PAH's, with only trace amounts of phenols, nitrogen- and sulfur-containing heterocyclic compounds, and heavy metals

(Saeed and Al-Mutairi 1999; Rodrigues et al. 2010). Small oil droplets are also part of WAF. The fate and transport of oil (and WAF) predominately involves dissolution, dispersion, photo-oxidation, biodegradation, sorption to and desorption from particulates (e.g., sediments, minerals, marine snow) (National Research Council (NRC) 2005, Fu et al. 2014; Overton et al. 2016). Dispersion and emulsification of oil may increase the bioavailability of oil products to biodegradation (NRC 2005); marine microbes play and important role in facilitating this activity.

The presence of oil or pyrogenic hydrocarbons and/or petrocarbons released during burning of oil impact aggregation rates and aggregate characteristics. Oil may be incorporated into aggregates during their formation, or collected when aggregates sink through oil plumes, or MOS may form via microbial response to oil (Passow 2016). Arnosti et al. (2016) and others (e.g., Azam 1998; Azam and Malfatti 2007; McGenity et al. 2012; Ziervogel et al. 2012) have described marine snow, including MOS, as "hot spots" for microbial organic matter and/or hydrocarbon degradation pathways in the ocean. Not only are they a location where petrocarbons and hydrocarbons are degraded but also where microbial biomass and EPS concentrations may be elevated (as shown in Fig. 3). Elevated hydrolytic enzyme activities were measured in the MOS during the DwH spill (Ziervogel et al. 2012; Arnosti et al. 2016) with a specific functionality that was different from those persisting in the surrounding seawater (Ziervogel et al. 2012; Arnosti et al. 2016). Patterns of polysaccharide-hydrolyzing enzyme activities were quite distinct between the individual members of microbial degradation networks, and differences in enzyme activities infer very distinct communities of microbes in the different micro-environments. Additionally, microbial communities in and around MOS changed over time (Arnosti et al. 2016), with communities first degrading the more bioavailable petroleum, then the secondary degradation products of the petroleum and microbial exudation products (Arnosti et al. 2016).

There appears to be direct (oxidizing, hydrolyzing, and assimilating) and indirect (emulsifying with EPS) participation by microbes in oil degradation pathways of MOS, as well as a primary and secondary set of degraders. Bacteria with genes for hydrocarbon degradation utilize or metabolize oil (Hazen et al. 2010; Prince et al. 2010; Valentine et al. 2010; Kessler et al. 2011; Lu et al. 2012; Redmond and Valentine 2012; Ziervogel et al. 2012). For example, members of the genus *Cycloclasticus* were found to directly degrade hydrocarbons during the DwH spill, while members of the genus *Halomonas* played an indirect role on aggregates (Arnosti et al. 2016). The activity of the primary degraders sets off a complex cascade of secondary degraders, including but not limited to those of aggregate-associated bacteria and free-living bacteria not incorporated into the aggregates. Free TEP and marine snow provide physical structure, allowing the development of such complex microbial

communities needed to degrade oil efficiently, similar to biofilms. The release of EPS by microbes as a response to petrocarbons also physically protects them, promotes attachment, and/or may serve to emulsify and solubilize oil products, thus increasing their bioavailability (Head et al. 2006; McGenity et al. 2012).

The production of EPS biosurfactants may be one important example of a mutualistic interaction between phytoplankton and bacteria in the presence of oil. Surfactants (surface active "soap-like" molecules) with hydrophilic and hydrophobic moieties (see Fig. 6) increase the bioavailability of certain oil components (McGenity et al. 2012). In this respect, these biosurfactant properties are similar to the dispersants used for oil spill remediation. Chemical surfactants are designed to reduce the interfacial tension between oil and water, thereby enhancing dispersion and potentially biodegradation processes (Lewis et al. 2010). Biosurfactants such as EPS or extracellular polysaccharides can emulsify petrocarbons (Head et al. 2006). EPS produced by *Halomonas* sp., for example, have amphiphilic properties, thereby interacting easily with hydrophobic substrates like hydrocarbons, leading to the solubilization and biodegradation of oil components (Gutierrez et al. 2013). The exopolymers with entrained oil droplets form networks that act as an energy and carbon source to other members of the microbial community.

The abundant, large (mm to cm), mucus-rich marine snow which appeared near the surface in the weeks after the DwH spill was produced by the microbial community in response to the released petrocarbons and hydrocarbons (Passow et al. 2012; Gutierrez et al. 2013; Ziervogel and Arnosti 2013). While some phytoplankton species are negatively impacted (e.g., Prouse et al. 1976; González et al. 2009; Hook and Osborn 2012; Ozhan et al. 2014a,b; Garr et al. 2015) others appear to thrive in the presence of oil (Prince et al. 2010; Almeda et al. 2014; Ozhan and Bargu 2014; Ozhan et al. 2014a,b), although it is unclear if phytoplankton benefit directly from oil, or via a symbiotic relationship with prokaryotes. Oil compounds may enter the food chain through bacteria (Graham et al. 2010; Chanton et al. 2012). Alternatively, we propose they may enter through marine snow or phytoplankton, both of which provides food for zooplankton. Food-web interactions will influence degradation rates of oil in a multitude of ways, such as bacterivorous protists that graze on the oil-consuming bacterial community potentially decreasing degradation rates (Beaudoin et al. 2016).

Experiments have revealed microbial MOS contains fossil carbon with the same ^{13}C signature as the oil (Passow 2016); this microbial MOS has a very different appearance from the aggregates formed due to coagulation of individual particles, like phytoplankton (see also Fig. 2 which provides an example). It consisted predominately of amorphous mucus, which was extremely sticky. It was suggested that in vivo

production of microbial mucus results in patches of "floating biofilm" which transforms into MOS once the material begins to sink below the air-oil-seawater interface. These too are aggregates with oil (MOS), but not the microbial MOS.

Diatom aggregates forming after the DwH spill incorporated oil, also formed sinking MOS, which was recovered in sediment traps at >1400 m (Passow 2016; Yan et al. 2016). Recent experimental evidence suggests the presence of oil increased the stability and cohesion of MOS, potentially leading to a faster and more efficient transport to sediments (Fu et al. 2014; Daly et al. 2016). Previous studies have found that in sediments phytoplankton is often closely associated with PAHs (Kowalewska and Konat 1997; Kowalewska 1999; Lubecki and Kowalewska 2010; Parsons 2014). The mechanisms leading to such an association were, however, largely unknown. Scavenging and inclusion of oil in phytoplankton aggregates is one potential mechanism (Alldredge and Gotschalk 1988, 1989). Alternatively, oil sorption to phytoplankton cells may also occur. Differences in the amount of oil incorporated into phytoplankton aggregates depends on the species (observed in lab studies), community composition (observed in field studies), cell packaging as well as the quantity and compositions of EPS (e.g., hydrophobic or hydrophilic) and types of oil (Passow 2016).

In Figs. 2–4, we show marine snow formed during mesocosm experiments performed with NGOM seawater "seeded" with microbial populations collected from Galveston Bay, Texas. Apart from controls, there were two treatments of (1) seawater amended water accommodated fraction (WAF) (Fig. 2B), and (2) seawater amended chemically enhanced WAF (CEWAF) prepared at two different concentrations (Fig. 2C,D). Results showed that despite higher EPS production, oil and/or dispersants preferentially partitioned into the colloidal and suspended particulate fractions rather than into the rapidly forming and sinking MOS (Chen Xu, unpubl.). When the seawater was ameliorated with WAF and CEWAF, we observed marine snow that was visibly and chemically different in composition, quality and quantity to the controls (Figs. 2, 4, respectively). We found that WAF and CEWAF had been incorporated into this material (particularly Fig. 2C), which is evident when micro-aggregates were viewed microscopically (Fig. 3C,D). As part of ongoing studies, this material was carefully characterized. A representative finding of the molecular characterization of the marine snow is given in Fig. 6. Initial analysis reveals that the EPS produced in response to CEWAF were more amphiphilic (higher protein content) and thus enhanced the dispersion of CEWAF and sedimentation of this material. The sedimented MOS contained about 30% of oil compounds, as determined through radiocarbon mass balance. On a cautionary note, these results would not necessarily be expected to apply to different conditions (such as near-shore vs offshore plankton community, nutrient levels, temperature, etc.).

Effects of dispersant on EPS-oil interactions

While the dispersant Corexit was primarily applied in response to the DwH oil spill; a mixture of SPC1000 and Mare-Clean200 were also used (The chaos of clean up (TCCP) 2011). Dispersant was added directly to the oil carpet at the sea surface as well as into the leak at various depths (Kujawinski et al. 2011). Dispersants are mixtures, containing as many as 57 different chemical ingredients that are partially soluble in both oil and water. Corexit consists of nonionic ($\sim$ 48%) and anionic ($\sim$ 35%) surfactants with enough solvent or petroleum distillate ($\sim$ 17%) to make a homogeneous dispersant mixture (Singer et al. 1991). When sprayed on an oil slick, dispersants enhance the dispersion of oil, e.g., its penetration onto and into the water. For example, Ramachandran et al. (2004) applied Corexit 9500 in a 1 : 50 ratio and found that it enhanced oil dispersion into the water by 68%. The increase was likely due to the presence of oil droplets in emulsion and the increased dissolution of petrocarbons and/or hydrocarbons from the surfaces of the numerous droplets (i.e., surface area effects). Increased dispersion is meant to lead to increased biodegradation of oil in water (Lewis et al. 2010), although this is currently controversial.

Corexit retarded, reduced, and/or completely inhibited the formation of MOS on a range of time scales, but response patterns vary (Passow 2016). The presence of Corexit may inhibit aggregation by dispersing micro-gels. Further, Corexit has been found to alter the microbial community responding to WAF by instead selecting for microorganisms that utilize Corexit as a substrate rather than oil (Kleindienst et al. 2015a,b). Other studies have shown the growth and viability of hydrocarbon-degrading microorganisms are reduced in the presence of Corexit (Hamdan and Fulmer 2011). In contrast, Baelum et al. (2012) observed that Corexit had no negative effects on microbial growth. These differences may be due to the respective microorganisms used in each study: Baelum et al. (2012) used natural deep water communities, while Hamdan and Fulmer (2011) used hydrocarbon degrading isolates. However, a host of other factors including oil type, weathering and composition, environmental conditions (temperature, pressure), and differences in EPS production and formation may also have played roles.

Surfactant behavior of EPS and dispersants

EPS may be characterized in terms of surfactant behavior in the presence of oil and dispersant using quantitative techniques. Effectiveness and efficiency of EPS surfactant behavior or that of chemical dispersants may be characterized through interfacial tension (IFT) and critical micelle concentration (CMC) rheology measurements. The *effectiveness* of EPS surfactant (or dispersing agent) is thought to be greater when a lower IFT initiates the CMC. When the CMC is reached at lower EPS concentrations, the *efficiency* of EPS as a

surfactant is greater. To demonstrate these concepts, we show EPS behaving as a biosurfactant in Fig. 6, that is, amphiphilic molecules (Fig. 6A) which, through intermolecular forces arrange their hydrophilic (anionic or non-ionic) polar head group in the water and their hydrophobic (non-polar) tail, which shun water, crossing the interfacial boundary to interact with the air or another hydrophobic substance, such as oil (Phase 1; Fig. 6). Amphiphilic EPS surfactant will form micelles (Fig. 6A,C), thereby dispersing hydrophobic molecules in the water (Fig. 6B).

The IFT is greatest in Phase 1 (Fig. 6), at the interface of the oil and seawater where there is little or no EPS. Amphiphilic molecules as part of EPS secreted by microbes diffuse to the interface (Phase 2; Fig. 6). The presence of these molecules on the surface disrupts the cohesive energy and lowers the IFT (Phase 2; Fig. 6). The purpose of surfactant or chemical dispersant application is to lower the oil/water IFT to promote entrainment of oil droplets into the water column and dispersion of the hydrophobic oil components (NRC 2005). When the surfactant concentration increases to the point that the oil/water liquid interface is saturated, the CMC is reached (between stages 2 and 3; Fig. 6). The concentration of dispersant at which the surfactant molecules form a uniform monolayer at the oil/water interface is the CMC (Phase 3; Fig. 6).

Micelles are generated from the EPS/surfactant molecules and the IFT can be decreased to the point that oil droplets are entrained in the micelles which range in size from ~ 3 nm to 50 nm (Phase 3; Fig. 6). It is within stage 3 that self-assembly occurs, wherein the EPS or dispersant surfactants interact with organic matter with the inclusion of water to reversibly form gels or aggregates (Fig. 6D), such as macrogels (~ 3–6 um), nanogels (~ 100–200 nm), TEP, or marine snow (Verdugo 2012). Micelles form with a roughly spherical shape (Fig. 6C). However, depending on the physico-chemical properties of the EPS/surfactant molecules, including the tail length and packing in the micelles, the micellar morphologies may be more cylindrical, laminar, cone-shaped, worm-like, and more (Jusufi et al. 2008), which probably accounts for the variance in morphology of TEP and marine snow from different microbial sources. The anionic moieties of the EPS head groups anneal through bridging with divalent cations prevalent in seawater, dominantly Ca^{2+} and Mg^{2+} ions, to form crystalized aggregate networks that increase the aggregate mass enough to sediment and may provide a mechanism to deter biodegradation (Chin et al. 1998) in addition to polymerization, and condensation of the size due to increasing pressure with depth (Chin et al. 1998).

Effect of reactive oxygen species on EPS production and oil interactions

Reactive oxygen species (ROS), have been found to lead to the production of EPS in natural conditions (Liu et al. 2007; Chen et al. 2009; Gong et al. 2012), and thus may potentially play a role in EPS-oil interactions. ROS includes singlet oxygen ($^1O_2^*$), superoxide radical ($\cdot O_2^-$), hydroxide radical ($\cdot OH$) and hydrogen peroxide (H_2O_2), and can be through metabolic and photochemical reactions (Kieber et al. 2003). ROS are capable of oxidizing a wide variety of compounds with relatively low selectivity, and can damage macromolecules including DNA, proteins and lipids. To relieve the oxidative stress of ROS, microbes excrete protective EPS to physically block or chemically quench the hazardous ROS (Liu et al. 2007; Chen et al. 2009; Gong et al. 2012). While ROS generated from high energy regions of visible light (UVB) assist in degrading macromolecules, ROS from light with somewhat lower energy (UVA) can crosslink macromolecules, e.g., proteins from marine bacteria (Sun et al., unpubl.) to form sinking particles. In this context, crosslinking refers to chemically binding to different regions of the macromolecule in a random fashion.

An imbalance between ROS and antioxidant production can lead to protein and lipid polymerization. This has been clearly demonstrated in the health sciences literature (e.g., plaques in the human brain and blood vessels, as reviewed in Valko et al. 2007). The polymerization may arise from cross-linking of radicals, which can be generated through ROS via triplet excited states of the biomolecules. A recent study has shown the proteins from marine bacteria may form aggregate via ROS during irradiation (Sun et al., unpubl.). Radical species that promote polymerization reactions can also be related to the presence of metals, such as Fe. Several studies on dissolved organic matter have shown that Fe takes part in the photo-flocculation process (Gao and Zepp 1998; Helms et al. 2013; Sun et al. 2014). Iron not only accelerates ROS production, such as the Fenton reaction, but can also bind to organic ligands as complexes (Chen et al. 2014; Sun and Mopper 2016). Recent studies by Waggoner et al. (2015) and Chen et al. (2014) have demonstrated, through molecular level analyses, that black-carbon like macromolecules can be formed from terrestrial dissolved organic matter in the presence of sunlight and Fe. Light effects on phytoplankton growth, EPS and ROS production occur simultaneously, and thus, would need to be distinguished to evaluate their effects on stickiness, aggregation, degradation and ultimately the fate of oil.

Additionally, ROS might accumulate during and after an oil spill under sunlight, and result in an increased oxidative stress to the microbes. A recent report showed that substantial amounts of $\cdot OH$ (1.2×10^{-16} to 2.4×10^{-16} M) was produced from the DwH spill and was at least one magnitude higher than concentrations of clean seawater (Ray and Tarr 2014). ROS may also play an important role in starting a biological cascade that induces the production of TEP. Diatoms undergoing oxidative stress produced large amounts of TEP following an induction of caspase (a family of enzymes implicated in triggering programmed cell death) activity (Kahl et al. 2008). High caspase activity also preceded a large

release of TEP in Fe-limited *Trichodesmium* cultures (Berman-Frank et al. 2007).

Effect of riverine input on EPS production and oil interactions

A freshwater diversion event (as a result of flood waters upstream) shortly after the DwH oil spill introduced significant quantities of not only freshwater but also nutrients, sediments and clay minerals into the NGOM (Hu et al. 2011; Gong et al. 2014; Walsh et al. 2015), all of which potentially affected productivity, EPS production and aggregation. Terrestrial sediment and clay mineral as well as resuspension of benthic materials in shallow near shore waters combined with oil may have led to the formation of oil-mineral aggregates (OMAs), or to the additional ballasting of marine snow (Muschenheim and Lee 2002; Khelifa et al. 2005; Niu et al. 2011; Gong et al. 2014; Passow 2016). OMAs have lower surface areas than oil droplets and hence undergo lower rates of biodegradation due to reduced oil availability. Nutrients, introduced from the Mississippi and Atchafalaya Rivers, may have led to enhanced productivity and potentially to increased EPS formation (Hu et al. 2011; Passow 2016). Alleviation of nutrient stress lasted a long time. In August 2010, 3 weeks after the oil well was capped, an area $> 11,000$ km^2 exhibited elevated chlorophyll concentrations, which were attributed directly and indirectly to the oil spill (Hu et al. 2011). It has been estimated via a modeling study that 12% of the biotic particle exported resulted from increased loadings of Mississippi River nutrients (Walsh et al. 2015).

Knowledge gaps

The microbial community and their EPS production can be viewed as the first responders to pollutants (oil, metals, nanomaterials) in the marine environment. EPS provides attachment (i.e., coagulation) to particle surfaces (biofilms, cell adhesion, marine snow flocs, MOSSFA), with the degree of attachment controlled directly or indirectly by the protein/carbohydrate ratio through reactions related to the surfactant behavior of EPS and ROS-mediated cross-linking reactions. Such interactions can be thought of as part of simultaneous processes occurring in a tightly balanced system that responds through biological and chemical interactions to any change in external or internal conditions (Varela et al. 1974; Maturana and Varela 1980; Santschi et al. 2003). Thus, EPS production by the microbial community and its composition is dynamic. This makes this system challenging to study because most approaches cannot easily distinguish between the biotic and chemical factors promoting the release of EPS. We suggest the following knowledge gaps that will need to be filled through interdisciplinary studies to better understand these complex systems:

1. *Operational definitions of EPS* (Table 1): It is still is not well understood how different operationally defined subgroups of EPS are related to each other (see also Bar-Zeev

et al. 2015). This ambiguous classification makes the task of identifying their relationships and interactions extremely complex and challenging.

2. *The best way to measure the surfactant nature, or amphiphilicity, of EPS* (see Tables 1, 2 for some initial details): It is not clear what measures best express the amphiphilic and surface-active nature of EPS: hydrophobic contact area, protein/carbohydrate ratio, aromaticity, surface charge, stainability with Alcian Blue or Coomassie blue.

3. *The best way to measure TEP*: Natural TEP consists of a varying mix of substances. For determination of TEP, historically the primary reference compound is Gum Xanthan, chosen because it looks and behaves more like TEP than other (e.g., Alginic Acid) tested compounds (Passow and Alldredge 1995). However, Gum Xanthan, which is derived from a terrestrial bacterium, does not occur in the ocean, while Alginic Acidis a common marine acidic polysaccharide (Guo et al. 2002; Hung et al. 2003; Santschi et al. 2003). The amount of TEP expressed as Gum Xanthan equivalents is up to a factor of 5 smaller than that expressed as Alginic Acid equivalents, depending on the relative ratios of carrageenan (sulfate groups), Alginic Acid (carboxyl groups), or other compounds, including phosphonates, as well as sample pretreatment (Hung et al. 2003). Consequently, the conversions from both, Gum Xanthan or Alginic Acid equivalents to TEP-C are hampered, because depending on the chemical composition of TEP the carbon content of TEP per Alcian Blue molecule varies and thus the conversion between TEP measured as Gum Xanthan or Alginic Acid equivalents and TEP-carbon is variable (Hung et al. 2003). As with EPS, the approach requires refinement.

4. *The relationship between stickiness and chemical properties of TEP*: TEP stickiness varies substantially and the relationship between stickiness and chemical properties of TEP are largely unidentified. We suggest that the protein/carbohydrate ratios can serve as a proxy for "stickiness," but it will still need to be shown how much relative hydrophobicity (through hydrophobic interactions) or ROS-mediated cross-linking (through chemical bond formation) contribute to the "stickiness" or aggregation potential leading to marine snow and MOSSFA in the presence of oil. We do know that Ca^{2+}-bridging is an important component for the formation of TEP—but we are not sure what and how this may impact stickiness.

5. *The environmental controls of microbial EPS production.* There remains a significant lack of understanding of why different microbes produce different types and amounts of EPS under different environmental conditions. Temperature, light, depth, mixing, pH, redox, are all external factors that can influence the formation of EPS and TEP by the marine biota.

6. *The relationships between EPS and the bacteria that degrade oil*: A complex network of microbes are using the

different components of oil and metabolites of the oil degraders will develop and facilitate biodegradation of the oil. Thus, while the presence of bacteria that can degrade the oil would indicate that they are also the originators of the EPS, such evidence is rather indirect.

7. *The relationship between phytoplankton-derived EPS and bacteria*: While there is evidence that bacteria attached to phytoplankton may be the major contributors to the EPS released in response to oil and Corexit pollution, the evidence is more indirect than direct, as different species can produce EPS without leaving a specific marker for its origin. Further, phytoplankton can produce TEP directly in the absence of bacteria; this exudation to TEP precursors is a physiological response to an imbalance in energy and carbon acquisition (Passow and Laws 2015). The details of these relationships need to be investigated. Understanding the complex relationship between phytoplankton and bacteria will help us to understand if they behave synergistically or antagonistically with regards to EPS release.

8. *The effects of light on phytoplankton growth, ROS, and EPS production and ultimately, on oil transformations*: Visible light, including UVB and UVA, produce different radical oxygen species in aquatic systems. In order relieve the oxidative stress of ROS, microbes can excrete protective EPS to physically block or chemically quench hazardous ROS. Contrary to UVB, which has a degrading effect on macromolecules, lower energy ROS generated from UVA appear to have the potential to cross-link proteins (e.g., in EPS), which has the potential to lead to EPS aggregation and marine snow formation. Since light effects phytoplankton growth, EPS and ROS production occur simultaneously, and their combined effects on the aggregation, degradation, and emulsification potential of EPS and subsequent fate and transport of oil needs to be evaluated.

9. *The relationship between dispersant and the microbial production of EPS and the microbial community composition*: Dispersants have been used to ameliorate the effects and transport of oil. It is however still not clear (1) how the presence of dispersants impacts the ability of bacteria and phytoplankton to generate EPS, (2) how the chemical dispersion of oil eliminates the stimulus that triggers the release of EPS, (3) how (and if) dispersants physically lead to the dispersion of EPS, and (4) how the presence of dispersants leads to a shift in the microbial composition towards strains less or more likely to generate mucus or degrade oil.

10. *The importance of riverine-derived terrestrial detrital matter and nutrients*: It is not clear to what extent the presence of riverine-derived terrestrial detrital material facilitates the formation and sinking of marine snow aggregates; the importance of seasonal timing, the magnitude or duration of the water discharge, and the composition of the discharge all need to be considered. We know for example, that the composition of the discharge varies significantly between the Mississippi and Atchafalaya Rivers despite both having similar source waters. This is thought to be because riverine waters from the former discharge directly into the NGOM while for the latter, the waters are processed in the estuary before heading to the NGOM. The differences in upstream biogeochemical processing have consequences on both the biology and chemistry once mixing with gulf waters occurs (see Quigg et al. 2011 and references therein) including but not limited to nutrient and light limitation of primary productivity. Further, it is not clear what the *role of nutrient stimulation after a spill* e.g., via the release of Mississippi River waters (but see Hu et al. 2011) has on the formation of EPS (marine snow).

Conclusions

In this paper, we focused on the importance of MOS, a biological process, as a transport pathway for oil to the seafloor. Previously, OMAs, tar balls, in addition to other non-biological processes have been considered in the sedimentation of oil. Floating oil residues (e.g., tar particles) were observed during the Ixtoc oil spill near Mexico in the GOM in 1979 (Patton et al. 1981; D'Souza et al. 2016). Ongoing studies (e.g., Daly et al. 2016) are finding indicators in deep sediments suggesting a MOSSFA event may have co-occurred during that spill as well. It is becoming increasingly clear that EPS and marine snow play an important role in determining the fate and transport of oil during and after a spill. This review highlights that there is relatively little known about EPS production in relation to oil and dispersants, especially how its amphiphilicity and surface activity are controlled at the molecular and chemical levels. When dispersants, which are artificial surfactants are used, the response of the microbial community has been even less studied (e.g., Gutierrez et al. 2013; Ziervogel and Arnosti 2013). There is much research remaining in order to develop better response strategies during oil spill events in future.

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